SELECTION OF MARKERS FOR MAPPING AND CLONING DISEASE RESISTANCE IN COMMON BEAN

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INTRODUCTION

Infestation of diseases is a major constraint of subsistence production and economic yield of common bean. Development of cultivars with improved genetic resistance to pest and diseases is the primary goal of bean breeding programs throughout the world.

DNA-marker-based genetic linkage maps have been developed and exploited to identify, tag, and map disease resistance genes and QTL in common bean. The availability of DNA-based markers within the past 20 years has provided new opportunities and challenges to bean researchers. While numerous molecular markers are available, especially for resistance to bacterial, fungal, and viral diseases in common bean, proportionately a few are routinely used in breeding programs. The recent development of SSRs from coding and non-coding sequences (Blair et al. 2003) and tentative consensus (TC) sequences (McConnell et al. 2006) in common bean have created further opportunities for mapping and tagging genes in breeding program.

Several classes of resistance genes encode proteins containing leucine-rich repeats (LRR). Amino acid sequences for a number of these resistance sequences contain strong similarity to nucleotide binding sites (NBS). We have analyzed NBS-LRR type disease resistance gene sequences in common bean and generated 37 molecular markers. In this study, our goal was to screen these markers along with 34 SSRs (Blair et al. 2003) and 21 markers developed from TC sequences (McConnell et al. 2006) among parents of seven mapping populations segregating for different disease resistance traits.

MATERIALS AND METHODS

Plant Materials: DNA was extracted from green house grown common bean parents of seven mapping populations using the protocol supplied by dry bean breeding group, department of plant sciences, North Dakota State University, Fargo, ND.

Development of PCR Primer: Common bean NBS-LRR type disease resistance gene sequences were downloaded from NCBI GenBank and were aligned with multiple sequence alignment software CLUSTALX (1.81). Phylogenetic trees were depicted to evaluate the relationship among different sequences using Neighbor-Joining options of the software. Initially, four NBS-LRR complete coding gene sequences were chosen to design a series of primers based on sequence alignment. The large (~3kb) gene sequences were divided into small ordered overlapping fragments and a total of 37 primer pairs were designed to amplify genomic DNA of ~500 bp, using the web-based PCR-primer designing program 'Primer3'. The primer design also ensured that there is sufficient overlap of the fragments, in order to obtain the sequence of the primer sites and their flanking nucleotides. In addition to these primers, 34 SSR primer pairs previously developed from coding and non-coding sequences of common bean (Blair et al. 2003) and 21 primer pairs developed by analyzing common bean tentative consensus (TC) sequences (McConnell et al., 2006) were also used.

Amplification of common bean genome: The screening of the primers developed from NBS-LRR gene sequences was performed using a PCR program consisting of one cycle of 95°C for 3 min; 40 cycles of 95°C for 1 min, from 55 to 57°C for 1 min, and 72°C for 2 min; and one cycle of 72°C for 10 min. The PCR program for SSR primers was consist one cycle of 95°C for 3 min; 40 cycles of 95°C for 1 min, from 55 to 57°C for 1 min, and 72°C for 2 min; and one cycle of 72°C for 10 min. For primers developed from TC sequences, the PCR protocol was one cycle of 95°C for 3 min. followed by 40 cycles of 95°C for 20 sec, from 52 to 58°C for 20 sec, and 72°C for 2 minutes. The PCR program ended with a 7 minutes cycle of 72°C for final extension. In all cases the amplified products were separated on 2% agarose gel with 60 volts and run for 200 minutes.

RESULTS

Forty-nine percent (49%) of these primers were polymorphic across different parental combination and markers developed from TCs were found to be highly polymorphic (76%) followed by SSRs (65%), and NBS-LRR types (27%). These primers showed varying degrees of polymorphism across different mapping populations. The highest number of primers (46) showed polymorphism in BAT93/JaloEEP558 mapping parent combination followed by A55/G122 (36) and Aztec/ND 88-104-04 (28) while the least polymorphism was observed in DOR364/XAN176 and Benton/NY6020-4 (each with 19 primers) (Fig. 1).

The map location of NBS-LRR type markers has yet to be determined. Sequencing of the amplified products from the NBS-LRR type primers across different parental lines is in progress. Population specific polymorphic markers selected here will be useful in mapping and tagging in different disease resistance traits.

LITERATURE CITED

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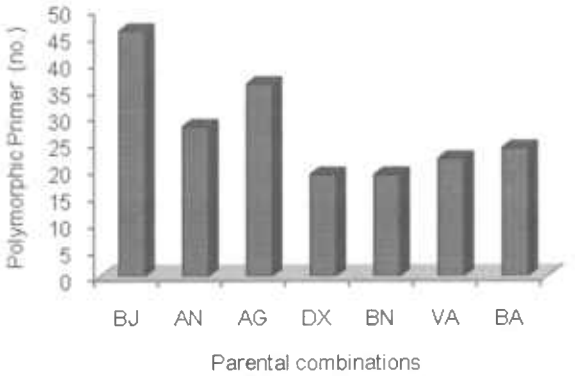

Fig. 1. Distribution of polymorphic primers among different parental combinations of mapping populations in common bean
BJ = BAT93/JaloEEP558, AN = Aztec/ ND88-104-04, AG = A55/G122, DX = DOR364/XAN176, BN = Benton/NY6020-4, VA = Voyager/Albion, and BA = BelNeb-RR-1/A55

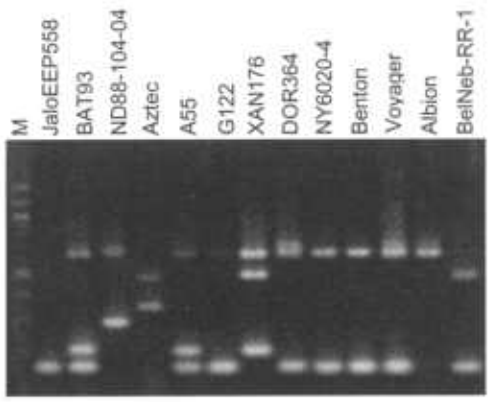

Fig. 2. Polymorphisms for NBS-LRR primer, Pv2356 among parental lines segregating for disease resistance traits